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(54) Title: HUMAN ALPHA$_4$ RECEPTOR SUBUNIT OF THE GABA-A RECEPTOR

(57) Abstract

The present invention relates to the cloning of novel cDNA sequences encoding the α_4 and δ receptor subunits of the human $GABA_A$ receptor; to stably co-transfected eukaryotic cell lines capable of expressing a human $GABA_A$ receptor, which receptor comprises at least one of the novel α_4 and δ receptor subunits; and to the use of such cell lines in screening for and designing medicaments which act upon the human $GAGA_A$ receptor.

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HUMAN ALPHA 4 RECEPTOR SUBUNIT OF THE GABA-A RECEPTOR

This invention concerns the cloning of a novel cDNA sequence encoding a particular subunit of the human $GABA_A$ receptor. In addition, the invention relates to a stable cell line capable of expressing said cDNA and to the use of the cell line in a screening technique for the design and development of subtype-specific medicaments.

Gamma-amino butyric acid (GABA) is a major inhibitory neurotransmitter in the central nervous system. It mediates fast synaptic inhibition by opening the chloride channel intrinsic to the $GABA_A$ receptor. This receptor comprises a multimeric protein of molecular size 230-270 kDa with specific binding sites for a variety of drugs including benzodiazepines, barbiturates and β-carbolines, in addition to sites for the agonist ligand GABA (for reviews see Stephenson, *Biochem. J.*, 1988, **249**, 21; Olsen and Tobin, *Faseb J.*, 1990, 4, 1469; and Sieghart, *Trends in Pharmacol. Sci.*, 1989, **10**, 407).

Molecular biological studies demonstrate that the receptor is composed of several distinct types of subunit, which are divided into four classes (α, β, γ and δ) based on their sequence similarities. To date, six types of α (Schofield *et al., Nature (London)*, 1987, **328**, 221; Levitan *et al., Nature (London)*, 1988, **335**, 76; Ymer *et al., EMBO J.*, 1989, 8, 1665; Pritchett & Seeberg, *J. Neurochem.*, 1990, 54, 802; Luddens *et al., Nature (London)*, 1990, **346**, 648; and Khrestchatisky *et al., Neuron*, 1989, **3**, 745), three types of β (Ymer *et al., EMBO J.*, 1989, 8, 1665), three types of γ (Ymer *et al., EMBO J.*, 1990, **9**, 3261; Shivers *et al., Neuron*, 1989, **3**, 327; and Knoflach *et al, FEBS Lett.*, 1991, **293**, 191) and one δ subunit (Shivers *et al., Neuron*, 1989, **3**, 327) have been identified.

The differential distribution of many of the subunits has been characterised by *in situ* hybridisation (Sequier *et al., Proc. Natl. Acad. Sci. USA*, 1988, **85**, 7815; Malherbe *et al., J. Neurosci.*, 1990, **10**, 2330; Shivers

et al., Neuron, 1989, 3, 327; and Wisden *et al, J. Neurosci.*, 1992, 12, 1040) and this has permitted it to be speculated which subunits, by their co-localisation, could theoretically exist in the same receptor complex.

Various combinations of subunits have been co-transfected into cells to identify synthetic combinations of subunits whose pharmacology parallels that of *bona fide* $GABA_A$ receptors *in vivo* (Pritchett *et al., Science*, 1989, **245**, 1389; Malherbe *et al., J. Neurosci.*, 1990, **10**, 2330; Pritchett and Seeberg, *J. Neurochem.*, 1990, **54**, 1802; and Luddens *et al., Nature (London)*, 1990, **346**, 648). This approach has revealed that, in addition to an α and β subunit, either γ_1 or γ_2 (Pritchett *et al., Nature (London)*, 1989, **338**, 582; Ymer *et al., EMBO J.*, 1990, **9**, 3261; and Malherbe *et al., J. Neurosci.*, 1990, **10**, 2330) or γ_3 (Herb *et al., Proc. Natl. Acad. Sci. USA*, 1992, **89**, 1433; Knoflach *et al., FEBS Lett.*, 1991, **293**, 191; and Wilson-Shaw *et al., FEBS Lett.*, 1991, **284**, 211) is also generally required to confer benzodiazepine sensitivity, and that the benzodiazepine pharmacology of the expressed receptor is largely dependent on the identity of the α and γ subunits present. Receptors containing a δ subunit (i.e. $\alpha\beta\delta$) do not appear to bind benzodiazepines (Shivers *et al., Neuron*, 1989, **3**, 327). Combinations of subunits have been identified which exhibit the pharmacological profile of a BZ_1 type receptor ($\alpha_1\beta_1\gamma_2$) and a BZ_2 type receptor ($\alpha_2\beta_1\gamma_2$ or $\alpha_3\beta_1\gamma_2$, Pritchett *et al., Nature (London)*, 1989, **338**, 582), as well as two $GABA_A$ receptors with a novel pharmacology, $\alpha_5\beta_2\gamma_2$ (Pritchett and Seeberg, *J. Neurochem.*, 1990, **54**, 1802) and $\alpha_6\beta_2\gamma_2$ (Luddens *et al., Nature (London)*, 1990, **346**, 648). Although the pharmacology of these expressed receptors appears similar to that of those identified in brain tissue by radioligand binding, it has nonetheless not been shown that these receptor subunit combinations exist *in vivo*.

A combination of subunits comprising either the human α_4 $GABA_A$ receptor subunit and/or the δ $GABA_A$ receptor subunit has not hitherto been possible due to the non-availability of the human α_4 cDNA or human

δ cDNA. This has consequently limited the use of cell lines in screening for subtype-specific medicaments, it being impossible to study the pharmacological profile of subunit combinations comprising the α_4 subunit and/or the δ subunit.

We have now ascertained the cDNA sequence of the α_4 subunit and the δ subunit of the human $GABA_A$ receptor. These nucleotide sequences, together with their deduced amino acid sequences corresponding thereto, are depicted in Figures 2 and 3 of the accompanying drawings.

The present invention accordingly provides, in a first aspect, a DNA molecule encoding the α_4 subunit of the human $GABA_A$ receptor comprising all or a portion of the sequence depicted in Figure 2, or a modified human sequence.

The present invention also provides, in another aspect, a DNA molecule encoding the δ subunit of the human $GABA_A$ receptor comprising all or a portion of the sequence depicted in Figure 3, or a modified human sequence.

The sequencing of the novel cDNA molecules in accordance with the invention can conveniently be carried out by the standard procedure described in accompanying Example 1; or may be accomplished by alternative molecular cloning techniques which are well known in the art, such as those described by Maniatis *et al.* in *Molecular Cloning, A Laboratory Manual*, Cold Spring Harbor Press, New York, 2nd edition, 1989.

In another aspect, the invention provides a recombinant expression vector comprising the nucleotide sequence of the human $GABA_A$ receptor α_4 subunit together with additional sequences capable of directing the synthesis of the said human $GABA_A$ receptor α_4 subunit in cultures of stably co-transfected eukaryotic cells.

The present invention also provides a recombinant expression vector comprising the nucleotide sequence of the human $GABA_A$ receptor δ subunit together with additional sequences capable of directing the

synthesis of the said human $GABA_A$ receptor δ subunit in cultures of stably co-transfected eukaryotic cells.

The term "expression vectors" as used herein refers to DNA sequences that are required for the transcription of cloned copies of recombinant DNA sequences or genes and the translation of their mRNAs in an appropriate host. Such vectors can be used to express eukaryotic genes in a variety of hosts such as bacteria, blue-green algae, yeast cells, insect cells, plant cells and animal cells. Specifically designed vectors allow the shuttling of DNA between bacteria-yeast, bacteria-plant or bacteria-animal cells. An appropriately constructed expression vector should contain: an origin of replication for autonomous replication in host cells, selective markers, a limited number of useful restriction enzyme sites, a high copy number, and strong promoters. A promoter is defined as a DNA sequence that directs RNA polymerase to bind to DNA and to initiate RNA synthesis. A strong promoter is one which causes mRNAs to be initiated at high frequency. Expression vectors may include, but are not limited to, cloning vectors, modified cloning vectors, specifically designed plasmids or viruses.

The term "cloning vector" as used herein refers to a DNA molecule, usually a small plasmid or bacteriophage DNA capable of self-replication in a host organism, and used to introduce a fragment of foreign DNA into a host cell. The foreign DNA combined with the vector DNA constitutes a recombinant DNA molecule which is derived from recombinant technology. Cloning vectors may include plasmids, bacteriophages, viruses and cosmids.

The recombinant expression vector in accordance with the invention may be prepared by inserting the nucleotide sequence of the $GABA_A$ α_4 subunit or the $GABA_A$ δ subunit into a suitable precursor expression vector (hereinafter referred to as the "precursor vector") using conventional recombinant DNA methodology known from the art. The precursor vector may be obtained commercially, or constructed by

standard techniques from known expression vectors. The precursor vector suitably contains a selection marker, typically an antibiotic resistance gene, such as the neomycin or ampicillin resistance gene. The precursor vector preferably contains a neomycin resistance gene, adjacent the SV40 early splicing and polyadenylation region; an ampicillin resistance gene; and an origin of replication, e.g. pBR322 ori. The vector also preferably contains an inducible promoter, such as MMTV-LTR (inducible with dexamethasone) or metallothionin (inducible with zinc), so that transcription can be controlled in the cell line of this invention. This reduces or avoids any problem of toxicity in the cells because of the chloride channel intrinsic to the $GABA_A$ receptor.

One suitable precursor vector is pMAMneo, available from Clontech Laboratories Inc. (Lee *et al., Nature*, 1981, **294**, 228; and Sardet *et al., Cell*, 1989, **56**, 271). Alternatively the precursor vector pMSGneo can be constructed from the vectors pMSG and pSV2neo.

The recombinant expression vector of the present invention is then produced by cloning the $GABA_A$ receptor α_4 subunit cDNA or the $GABA_A$ receptor δ subunit cDNA into the above precursor vector. The receptor subunit cDNA is subcloned from the vector in which it is harboured, and ligated into a restriction enzyme site, e.g. the Hind III site, in the polylinker of the precursor vector, for example pMAMneo or pMSGneo, by standard cloning methodology known from the art, and in particular by techniques analogous to those described herein. Before this subcloning, it is often advantageous, in order to improve expression, to modify the end of the α_4 or δ subunit cDNA with additional 5' untranslated sequences, for example by modifying the 5' end of the α_4 or δ subunit DNA by addition of 5' untranslated region sequences from the α_1 subunit DNA.

One suitable expression vector of the present invention is illustrated in Fig. 1 of the accompanying drawings, in which R represents the nucleotide sequence of the α_4 or δ subunit of the $GABA_A$ receptor, and

the remainder of the expression vector depicted therein is derived from the precursor vector pMSGneo.

According to a further aspect of the present invention, there is provided a stably co-transfected eukaryotic cell line capable of expressing a $GABA_A$ receptor, which receptor comprises the alpha-4 receptor subunit, at least one beta receptor subunit and the delta receptor subunit.

In another aspect of the present invention, there is provided a stably co-transfected eukaryotic cell line capable of expressing a $GABA_A$ receptor, which receptor comprises the alpha-4 receptor subunit, at least one beta receptor subunit and at least one gamma receptor subunit.

In a further aspect of the present invention, there is provided a stably co-transfected eukaryotic cell line capable of expressing a $GABA_A$ receptor, which receptor comprises at least one alpha receptor subunit, at least one beta receptor subunit and the delta receptor subunit.

This is achieved by co-transfecting cells with three expression vectors, each harbouring cDNAs encoding for an α_4, β or δ $GABA_A$ receptor subunit, or for an α_4, β or γ $GABA_A$ receptor subunit, or for an α, β or δ $GABA_A$ receptor subunit. In a further aspect, therefore, the present invention provides a process for the preparation of a eukaryotic cell line capable of expressing a $GABA_A$ receptor, which comprises stably co-transfecting a eukaryotic host cell with at least three expression vectors, one such vector harbouring the cDNA sequence encoding the α_4 $GABA_A$ receptor subunit another such vector harbouring the cDNA sequence encoding a beta $GABA_A$ receptor subunit, and a third such vector harbouring the cDNA sequence encoding the delta $GABA_A$ receptor subunit. The stable cell-line which is established expresses an $\alpha_4\beta\delta$ $GABA_A$ receptor.

The present invention also provides a process for the preparation of a eukaryotic cell line capable of expressing a $GABA_A$ receptor, which comprises stably co-transfecting a eukaryotic host cell with at least three expression vectors, one such vector harbouring the cDNA sequence

encoding the α_4 $GABA_A$ receptor subunit another such vector harbouring the cDNA sequence encoding a beta $GABA_A$ receptor subunit, and a third such vector harbouring the cDNA sequence encoding a gamma $GABA_A$ receptor subunit. The stable cell-line which is established expresses an $\alpha_4\beta\gamma$ $GABA_A$ receptor.

Similarly, the present invention provides a process for the preparation of a eukaryotic cell line capable of expressing a $GABA_A$ receptor, which comprises co-transfecting a eukaryotic host cell with at least three expression vectors, one such vector harbouring the cDNA sequence encoding an alpha $GABA_A$ receptor subunit, another such vector harbouring the cDNA sequence encoding a beta $GABA_A$ receptor subunit, and a third such vector harbouring the cDNA sequence encoding the δ $GABA_A$ receptor subunit. The stable cell line which is established expresses an $\alpha\beta\delta$ $GABA_A$ receptor.

Each receptor thereby expressed, comprising a unique combination of α_4, β and δ subunits, or α_4, β and γ subunits, or α, β and δ subunits, will be referred to hereinafter as a $GABA_A$ receptor "subunit combination". Pharmacological and electrophysiological data confirm that the recombinant $\alpha_4\beta\gamma$ receptor expressed by the cells of the present invention has the properties expected of a native $GABA_A$ receptor.

Expression of the $GABA_A$ receptor may be accomplished by a variety of different promoter-expression systems in a variety of different host cells. The eukaryotic host cells suitably include yeast, insect and mammalian cells. Preferably the eukaryotic cells which can provide the host for the expression of the receptor are mammalian cells. Suitable host cells include rodent fibroblast lines, for example mouse Ltk^-, Chinese hamster ovary (CHO) and baby hamster kidney (BHK); HeLa; and HEK293 cells. It is necessary to incorporate the α_4 subunit, at least one β and the δ subunit into the cell line in order to produce the required receptor, or alternatively the α_4 subunit and at least one β and one γ subunit or alternatively at least one α, one β and the δ subunit. Within

this limitation, the choice of receptor subunit combination is made according to the type of activity or selectivity which is being screened for.

In order to employ this invention most effectively for screening purposes, it is preferable to build up a library of cell lines, each with a different combination of subunits. Typically a library of 5 or 6 cell line types is convenient for this purpose. Preferred subunit combinations include: $\alpha_4\beta_3\gamma_2$, $\alpha_4\beta_3\delta$ and $\alpha_6\beta_3\delta$. Another preferred subunit combination is $\alpha_4\beta_2\gamma_2$.

As stated above, for each cell line of the present invention, three such vectors will be necessary, one containing the α_4 subunit, one containing a β subunit, and the third containing the δ subunit, or alternatively, one containing the α_4 subunit, one containing a β subunit, and the third containing a γ subunit, or alternatively, one containing an α subunit, one containing a β subunit and one containing the δ subunit.

Cells are then co-transfected with the desired combination of three expression vectors. There are several commonly used techniques for transfection of eukaryotic cells *in vitro*. Calcium phosphate precipitation of DNA is most commonly used (Bachetti *et al.*, *Proc. Natl. Acad. Sci. USA*, 1977, 74, 1590-1594; Maitland *et al.*, *Cell*, 1977, 14, 133-141), and represents a favoured technique in the context of the present invention.

A small percentage of the host cells takes up the recombinant DNA. In a small percentage of those, the DNA will integrate into the host cell chromosome. Because the neomycin resistance gene will have been incorporated into these host cells, they can be selected by isolating the individual clones which will grow in the presence of neomycin. Each such clone is then tested to identify those which will produce the receptor. This is achieved by inducing the production, for example with dexamethasone, and then detecting the presence of receptor by means of radioligand binding.

In a further aspect, the present invention provides protein preparations of $GABA_A$ receptor subunit combinations, especially human

$GABA_A$ receptor subunit combinations, derived from cultures of stably transfected eukaryotic cells. The invention also provides preparations of membranes containing subunit combinations of the $GABA_A$ receptor, especially human $GABA_A$ receptor subunit combinations, derived from cultures of stably transfected eukaryotic cells.

The cell line, and the membrane preparations therefrom, according to the present invention have utility in screening and design of drugs which act upon the $GABA_A$ receptor, for example benzodiazepines, barbiturates, β-carbolines and neurosteroids. The present invention accordingly provides the use of the cell line described above, and membrane preparations derived therefrom, in screening for and designing medicaments which act upon the $GABA_A$ receptor. Of particular interest in this context are molecules capable of interacting selectively with $GABA_A$ receptors made up of varying subunit combinations. As will be readily apparent, the cell line in accordance with the present invention, and the membrane preparations derived therefrom, provide ideal systems for the study of structure, pharmacology and function of the various $GABA_A$ receptor subtypes.

The following non-limiting Examples illustrate the present invention.

EXAMPLE 1

ISOLATION AND SEQUENCING OF cDNAS ENCODING THE HUMAN $GABA_A$ RECEPTOR α_4 SUBUNIT

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a) cDNA libraries

cDNAs were cloned from human foetal brain and adult hippocampus cDNA libraries. All cDNA libraries were constructed in the lambdaZAP vector, and were purchased from Stratagene (San Diego,

California). For screening, the cDNA libraries were plated according to the manufacturer's instructions, at 40,000 pfu per 137 mm plate. Filter lifts were taken using Hybond N filters (Amersham) according to the manufacturer's instructions.

b) Isolation of cDNA encoding human α_4 subunit

A human α_4 probe was first generated by polymerase chain reaction (PCR) using oligonucleotide primers (synthesised on an Applied Biosystems 380B synthesizer) derived from the bovine α_4 sequence (Ymer *et al, FEBS Lett.*, 1989, 258, 119): 5'TTTCAGGAATTCCAGTGCTGAGAGAAAAGCATCCTGAAAC3' (bp 1121-1160, containing an EcoRI restriction enzyme site) SEQ. ID. NO.:1, and 5'ATCCAGAAGCTTGTGGAGCAGAGGGAGTAGTAGTGGC3' (antisense, bp 1540-1577, incorporating a HindIII restriction enzyme site) SEQ. ID. NO.:2. PCR was performed as described, for example, by Whiting *et al* in *Proc. Natl. Acad. Sci., USA*, 1990, 87, 9966, using a human foetal brain cDNA library as a template. The PCR product was digested with EcoRI and HindIII and subcloned into similarly digested pBluescript SK- and its identity confirmed by DNA sequencing using standard techniques and the Sequenase II enzyme (United States Biochemicals).

A human foetal brain cDNA library was screened using ^{32}P labelled human α_4 probe DNA as described above. A single cDNA clone, approximately 2500bp, was obtained. DNA sequencing indicated that this cDNA clone contained 3' untranslated sequences and 3' coding region up to bp 1162 of the bovine cDNA sequence. The missing 5' sequence was obtained by anchored PCR using human brain 5'-RACE-Ready cDNA (CLONTECH, Palo Alto, CA), according to the manufacturer's instructions. The antisense oligonucleotides used for nested PCR were 5'ATTGGCATTTGTATTCTGCAGAGG3' SEQ. ID. NO.:3, and 5'GGAAGATTTGCTTGAATGGTTTGG3' SEQ. ID. NO.:4. A 1200bp PCR

product was obtained. DNA sequencing confirmed that this cDNA contained the missing 5' sequence of the α_4 cDNA, extending to 130bp 5' of the initiating ATG codon.

A full length α_4 cDNA was generated by PCR using oligonucleotide primers generated from sequences of the 5' and 3' untranslated region: 5' sense primer 5'CCTGGATCCGTGAACAGGCTTGAAGTATG3' (incorporating a BamHI restriction enzyme site) SEQ. ID. NO.:5; 3' antisense primer 5'ACGAATTCACATTAGACTTTCTGATTTCTC3' (incorporating an EcoRI restriction enzyme site) SEQ. ID. NO.:6. PCR was performed using human brain thalamus cDNA. A 1500bp product was generated which was subcloned into the cloning/eukaryotic expression vector pcDNA/Amp (Invitrogen). The cDNA was sequenced completely on both strands using an Applied Biosystems 373A DNA sequencer and dye terminator chemistry according to the manufacturer's instructions.

The complete nucleotide sequence of the cDNA encoding the human α_4 subunit, together with the deduced amino acid sequence corresponding thereto is shown in Fig. 2 of the accompanying drawings SEQ. ID. NOS.:7 and 8.

EXAMPLE 2

ISOLATION AND SEQUENCING OF cDNAS ENCODING THE HUMAN $GABA_A$ RECEPTOR δ SUBUNIT

---

a) cDNA libraries

As described in Example 1(a).

b) Isolation of cDNA encoding human δ subunit

A rat δ subunit probe was first generated by PCR using oligonucleotide primers derived from the rat δ subunit sequence (Shivers

et al, Neuron, 1989, 3, 327):
5'AGCCCGAATTCCATGGACGTTCTGGGCTGGCTG3' (bp 18-51, incorporating an EcoRI restriction enzyme site) SEQ. ID. NO.:9 and 5'GGTTTCCAAGCTTACTTTGGAGAGGTAGC3' (bp 1357-1390, incorporating a HindIII restriction enzyme site) SEQ. ID. NO.:10. PCR was performed as described, for example, by Whiting *et al, Proc. Natl. Acad. Sci., USA,* 1990, 87, 9966, using rat brain cDNA as template. A 1400bp product was obtained, subcloned into pBluescript SK- and its identity confirmed by DNA sequencing. A human hippocampus cDNA library was screened using ^{32}P labelled rat δ subunit probe DNA as described above. A single clone was obtained containing an 1800bp insert. DNA sequencing indicated that this cDNA contained the complete coding region of the human δ subunit. The cDNA was sequenced completely on both strands using an Applied Biosystems 373A DNA sequencer and dye terminator chemistry according to the manufacturer's instructions.

The complete nucleotide sequence of the cDNA encoding the human δ subunit, together with the deduced amino acid sequence corresponding thereto is shown in Fig. 3 of the accompanying drawings SEQ. ID. NOS.:11 and 12.

EXAMPLE 3

EXPRESSION OF HUMAN α_4 cDNA IN *XENOPUS* OOCYTES

The human α_4 cDNA (Example 1, Fig. 2) was subcloned into the eukaryotic expresion vector, pCDNA I Amp (Invitrogen, San Diego CA). Expression of this cDNA was investigated using the *Xenopus* oocyte system. Methods for preparation of *Xenopus* oocytes, nuclear injection of cDNAs, and eletrophysiological recordings from oocytes expressing

recombinant $GABA_A$ receptors, are well documented (see, for instance, Hadingham *et al., Mol. Pharmacol.*, 1993, 44, 1211-1218).

When co-expressed with β_2 and γ_2 cDNAs (Hadingham *et al., Mol. Pharmacol.*, 1993, 44, 1211-1218) minimal expressed of $GABA_A$ gated chloride currents were observed (10-50nA whole cell currents as measured under voltage clamped conditions). To increase the efficiency of expression the α_4 cDNA was re-engineered so as to replace the 5' untranslated sequence and signal peptide with the corresponding α_1 sequence. PCR was performed using the α_1 cDNA (Schofield *et al., Nature (London)*, 1987, 328, 221) as template. Primers were (i) 5'TAATGAGTTTAAACCATAGCTTCTTCCAGT3' (bp12-35 of α_1 incorporating a BamHI site) SEQ. ID. NO. :11, and (ii) 5'CATGATGGATCCGCCCGCTCAGAC3' (bp 269-305 incorporating a PmeI site) SEQ. ID. NO.:12. The BamHI-PmeI cut PCR fragment was subcloned into similarly cut α_4 pCDNA I Amp. When this α_4 construct was co-expressed in *Xenopus* oocytes with β_2 and γ_2 cDNAs robust $GABA_A$ gated currents of up to 1000nA whole cell current were obtained.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:

(A) NAME: Merck Sharp & Dohme Limited

(B) STREET: Terlings Park

(C) CITY: Harlow

(D) STATE: Essex

(E) COUNTRY: England

(F) POSTAL CODE (ZIP): CM20 2QR

(ii) TITLE OF INVENTION: Novel Cloned GABA-A Receptor Subunit cDNA Sequences and Stably Co-transfected Cell Lines

(iii) NUMBER OF SEQUENCES: 14

(iv) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk

(B) COMPUTER: IBM PC compatible

(C) OPERATING SYSTEM: PC-DOS/MS-DOS

(D) SOFTWARE: PatentIn Release #1.0, Version #1.25 (EPO)

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 40 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

TTTCAGGAAT TCCAGTGCTG AGAGAAAAGC ATCCTGAAAC

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 237 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

ATCCAGAAGC TTGTGGAGCA GAGGGAGTAG TAGTGGC

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

ATTGGCATTT GTATTCTGCA GAGG

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

GGAAGATTTG CTTGAATGGT TTGG

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 29 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

CCTGGATCCG TGAACAGGCT TGAAGTATG

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

ACGAATTCAC ATTAGACTTT CTGATTTCTC

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1707 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 39..1703

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

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        10          20          30          41          50          59
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GGATCCGTGA ACAGCTTGAA GTATGGCATG TTGCAAAG ATG GTT TCT GCC AAG AAG GTA
                                          MET Val Ser Ala Lys Lys Val

         68          77          86          95          104         113
CCC GCG ATC ACT CTG TCC GCC GGG GTC AGT TTC GCC CTC CTG CGC TTC CTG TGC
Pro Ala Ile Thr Leu Ser Ala Gly Val Ser Phe Ala Leu Leu Arg Phe Leu Cys

        122         131         140         149         158         167
CTG GCG GTT TGT TTA AAC GAA TCC CCA GGA CAG AAC CAA AAG GAG GAG AAA TTG
Leu Ala Val Cys Leu Asn Glu Ser Pro Gly Gln Asn Gln Lys Glu Glu Lys Leu

        176         185         194         203         212         221
TGC ACA GAA AAT TTC ACC CGC ATC CTG GAC AGT TTG CTC GAT GGT TAT GAC AAC
Cys Thr Glu Asn Phe Thr Arg Ile Leu Asp Ser Leu Leu Asp Gly Tyr Asp Asn

        230         239         248         257         266         275
AGG CTG CGT CCT GGA TTT GGG GGT CCT GTT ACA GAA GTG AAA ACT GAC ATA TAT
Arg Leu Arg Pro Gly Phe Gly Gly Pro Val Thr Glu Val Lys Thr Asp Ile Tyr

        284         293         302         311         320         329
GTC ACC AGC TTT GGA CCT GTT TCT GAT GTT GAA GTG GAA TAC ACA ATG GAT GTG
Val Thr Ser Phe Gly Pro Val Ser Asp Val Glu Val Glu Tyr Thr MET Asp Val

        338         347         356         365         374         383
TTC TTC AGG CAG ACA TGG ATT GAC AAA AGA TTA AAA TAT GAC GGC CCC ATT GAA
Phe Phe Arg Gln Thr Trp Ile Asp Lys Arg Leu Lys Tyr Asp Gly Pro Ile Glu

        392         401         410         419         428         437
ATT TTG AGA TTG AAC AAT ATG ATG GTA ACG AAA GTG TGG ACC CCT GAT ACT TTC
Ile Leu Arg Leu Asn Asn MET MET Val Thr Lys Val Trp Thr Pro Asp Thr Phe

        446         455         464         473         482         491
TTC AGG AAT GGA AAG AAA TCT GTC TCA CAT AAT ATG ACA GCT CCA AAT AAG CTT
Phe Arg Asn Gly Lys Lys Ser Val Ser His Asn MET Thr Ala Pro Asn Lys Leu

        500         509         518         527         536         545
TTT AGA ATT ATG AGA AAT GGT ACT ATT TTA TAC ACA ATG AGA CTC ACC ATA AGT
Phe Arg Ile MET Arg Asn Gly Thr Ile Leu Tyr Thr MET Arg Leu Thr Ile Ser
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        554         563         572         581         590         599
GCG GAG TGT CCC ATG AGA TTG GTG GAT TTT CCC ATG GAT GGT CAT GCA TGC CCT
Ala Glu Cys Pro MET Arg Leu Val Asp Phe Pro MET Asp Gly His Ala Cys Pro

        608         617         626         635         644         653
GTG AAA TTC GGG AGT TAT GCC TAT CCA AAG AGT GAG ATG ATC TAT ACC TGG ACA
Val Lys Phe Gly Ser Tyr Ala Tyr Pro Lys Ser Glu MET Ile Tyr Thr Trp Thr

        662         671         680         689         698         707
AAA GGT CCT GAG AAA TCA GTT GAA GTT CCG AAG GAG TCT TCC AGC TTA GTT CAA
Lys Gly Pro Glu Lys Ser Val Glu Val Pro Lys Glu Ser Ser Ser Leu Val Gln

        716         725         734         743         752         761
TAT GAT TTG ATT GGG CAA ACC GTA TCA AGT GAA ACC ATC AAA TCA ATT ACG GGT
Tyr Asp Leu Ile Gly Gln Thr Val Ser Ser Glu Thr Ile Lys Ser Ile Thr Gly

        770         779         788         797         806         815
GAA TAT ATT GTT ATG ACG GTT TAC TTC CAC CTC AGA CGG AAG ATG GGT TAT TTT
Glu Tyr Ile Val MET Thr Val Tyr Phe His Leu Arg Arg Lys MET Gly Tyr Phe

        824         833         842         851         860         869
ATG ATT CAG ACC TAT ATT CCG TGC ATT ATG ACA GTG ATT CTT TCT CAA GTT TCA
MET Ile Gln Thr Tyr Ile Pro Cys Ile MET Thr Val Ile Leu Ser Gln Val Ser

        878         887         896         905         914         923
TTT TGG ATA AAT AAA GAA TCA GTT CCC GCT AGG ACC GTA TTT GGA ATA ACA ACT
Phe Trp Ile Asn Lys Glu Ser Val Pro Ala Arg Thr Val Phe Gly Ile Thr Thr

        932         941         950         959         968         977
GTC CTC ACC ATG ACC ACA CTA AGC ATC AGT GCA CGA CAT TCT TTG CCC AAA GTG
Val Leu Thr MET Thr Thr Leu Ser Ile Ser Ala Arg His Ser Leu Pro Lys Val

        986         995        1004        1013        1022        1031
TCC TAT GCT ACC GCC ATG GAC TGG TTC ATA GCT GTC TGC TTT GCT TTT GTA TTT
Ser Tyr Ala Thr Ala MET Asp Trp Phe Ile Ala Val Cys Phe Ala Phe Val Phe

       1040        1049        1058        1067        1076        1085
TCG GCC CTT ATC GAG TTT GCT GCT GTC AAC TAT TTC ACC AAT ATT CAA ATG GAA
Ser Ala Leu Ile Glu Phe Ala Ala Val Asn Tyr Phe Thr Asn Ile Gln MET Glu

       1094        1103        1112        1121        1130        1139
AAA GCC AAA AGG AAG ACA TCA AAG CCC CCT CAG GAA GTT CCC GCT GCT CCA GTG
Lys Ala Lys Arg Lys Thr Ser Lys Pro Pro Gln Glu Val Pro Ala Ala Pro Val

       1148        1157        1166        1175        1184        1193
CAG AGA GAG AAG CAT CCT GAA GCC CCT CTG CAG AAT ACA AAT GCC AAT TTG AAC
Gln Arg Glu Lys His Pro Glu Ala Pro Leu Gln Asn Thr Asn Ala Asn Leu Asn

       1202        1211        1220        1229        1238        1247
ATG AGA AAA AGA ACA AAT GCT TTG GTT CAC TCT GAA TCT GAT GTT GGC AAC AGA
MET Arg Lys Arg Thr Asn Ala Leu Val His Ser Glu Ser Asp Val Gly Asn Arg
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      1256        1265        1274        1283        1292        1301
ACT GAG GTG GGA AAC CAT TCA AGC AAA TCT TCC ACA GTT GTT CAA GAA TCT TCT
Thr Glu Val Gly Asn His Ser Ser Lys Ser Ser Thr Val Val Gln Glu Ser Ser

      1310        1319        1328        1337        1346        1355
AAA GGC ACA CCT CGG TCT TAC TTA GCT TCC AGT CCA AAC CCA TTC AGC CGT GCA
Lys Gly Thr Pro Arg Ser Tyr Leu Ala Ser Ser Pro Asn Pro Phe Ser Arg Ala

      1364        1373        1382        1391        1400        1409
AAT GCA GCT GAA ACC ATA TCT GCA GCA AGA GCA CTT CCA TCT GCT TCT CCT ACT
Asn Ala Ala Glu Thr Ile Ser Ala Ala Arg Ala Leu Pro Ser Ala Ser Pro Thr

      1418        1427        1436        1445        1454        1463
TCT ATC CGA ACT GGA TAT ATG CCT CGA AAG GCT TCA GTT GGA TCT GCT TCT ACT
Ser Ile Arg Thr Gly Tyr MET Pro Arg Lys Ala Ser Val Gly Ser Ala Ser Thr

      1472        1481        1490        1499        1508        1517
CGT CAC GTG TTT GGA TCA AGA CTG CAG AGG ATA AAG ACC ACA GTT AAT ACC ATA
Arg His Val Phe Gly Ser Arg Leu Gln Arg Ile Lys Thr Thr Val Asn Thr Ile

      1526        1535        1544        1553        1562        1571
GGG GCT ACT GGG AAG TTG TCA GCT ACT CCT CCT CCA TCG GCT CCA CCA CCT TCT
Gly Ala Thr Gly Lys Leu Ser Ala Thr Pro Pro Pro Ser Ala Pro Pro Pro Ser

      1580        1589        1598        1607        1616        1625
GGA TCT GGC ACA AGT AAA ATA GAC AAA TAT GCC CGT ATT CTC TTT CCA GTC ACA
Gly Ser Gly Thr Ser Lys Ile Asp Lys Tyr Ala Arg Ile Leu Phe Pro Val Thr

      1634        1643        1652        1661        1670        1679
TTT GGG GCA TTT AAC ATG GTT TAT TGG GTT GTT TAT TTA TCT AAG GAC ACT ATG
Phe Gly Ala Phe Asn MET Val Tyr Trp Val Val Tyr Leu Ser Lys Asp Thr MET

      1688        1697        1707
                             >
GAG AAA TCA GAA AGT CTA ATG TGA ATTC
Glu Lys Ser Glu Ser Leu MET
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(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 554 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

MET Val Ser Ala Lys Lys Val Pro Ala Ile Thr Leu Ser Ala Gly Val Ser Phe Ala
1 5 10 15

Leu Leu Arg Phe Leu Cys Leu Ala Val Cys Leu Asn Glu Ser Pro Gly Gln Asn Gln
20 25 30 35

Lys Glu Glu Lys Leu Cys Thr Glu Asn Phe Thr Arg Ile Leu Asp Ser Leu Leu Asp
40 45 50 55

Gly Tyr Asp Asn Arg Leu Arg Pro Gly Phe Gly Gly Pro Val Thr Glu Val Lys Thr
60 65 70 75

Asp Ile Tyr Val Thr Ser Phe Gly Pro Val Ser Asp Val Glu Val Glu Tyr Thr MET
80 85 90 95

Asp Val Phe Phe Arg Gln Thr Trp Ile Asp Lys Arg Leu Lys Tyr Asp Gly Pro Ile
100 105 110

Glu Ile Leu Arg Leu Asn Asn MET MET Val Thr Lys Val Trp Thr Pro Asp Thr Phe
115 120 125 130

Phe Arg Asn Gly Lys Lys Ser Val Ser His Asn MET Thr Ala Pro Asn Lys Leu
135 140 145 150

Phe Arg Ile MET Arg Asn Gly Thr Ile Leu Tyr Thr MET Arg Leu Thr Ile Ser
155 160 165

Ala Glu Cys Pro MET Arg Leu Val Asp Phe Pro MET Asp Gly His Ala Cys Pro
170 175 180 185

Val Lys Phe Gly Ser Tyr Ala Tyr Pro Lys Ser Glu MET Ile Tyr Thr Trp Thr
190 195 200 205

Lys Gly Pro Glu Lys Ser Val Glu Val Pro Lys Glu Ser Ser Ser Leu Val Gln
210 215 220

Tyr Asp Leu Ile Gly Gln Thr Val Ser Ser Glu Thr Ile Lys Ser Ile Thr Gly
225 230 235 240

Glu Tyr Ile Val MET Thr Val Tyr Phe His Leu Arg Arg Lys MET Gly Tyr Phe
245 250 255

MET Ile Gln Thr Tyr Ile Pro Cys Ile MET Thr Val Ile Leu Ser Gln Val Ser
260 265 270 275

Phe Trp Ile Asn Lys Glu Ser Val Pro Ala Arg Thr Val Phe Gly Ile Thr Thr
280 285 290 295

Val Leu Thr MET Thr Thr Leu Ser Ile Ser Ala Arg His Ser Leu Pro Lys Val
300 305 310

Ser Tyr Ala Thr Ala MET Asp Trp Phe Ile Ala Val Cys Phe Ala Phe Val Phe
315 320 325 330

Ser Ala Leu Ile Glu Phe Ala Ala Val Asn Tyr Phe Thr Asn Ile Gln MET Glu
335 340 345

Lys Ala Lys Arg Lys Thr Ser Lys Pro Pro Gln Glu Val Pro Ala Ala Pro Val

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350                 355                 360                 365

Gln Arg Glu Lys His Pro Glu Ala Pro Leu Gln Asn Thr Asn Ala Asn Leu Asn
        370                 375                 380                 385

MET Arg Lys Arg Thr Asn Ala Leu Val His Ser Glu Ser Asp Val Gly Asn Arg
                390                 395                 400

Thr Glu Val Gly Asn His Ser Ser Lys Ser Ser Thr Val Val Gln Glu Ser Ser
    405                 410                 415                 420

Lys Gly Thr Pro Arg Ser Tyr Leu Ala Ser Ser Pro Asn Pro Phe Ser Arg Ala
            425                 430                 435

Asn Ala Ala Glu Thr Ile Ser Ala Ala Arg Ala Leu Pro Ser Ala Ser Pro Thr
440                 445                 450                 455

Ser Ile Arg Thr Gly Tyr MET Pro Arg Lys Ala Ser Val Gly Ser Ala Ser Thr
        460                 465                 470                 475

Arg His Val Phe Gly Ser Arg Leu Gln Arg Ile Lys Thr Thr Val Asn Thr Ile
                480                 485                 490

Gly Ala Thr Gly Lys Leu Ser Ala Thr Pro Pro Pro Ser Ala Pro Pro Pro Ser
    495                 500                 505                 510

Gly Ser Gly Thr Ser Lys Ile Asp Lys Tyr Ala Arg Ile Leu Phe Pro Val Thr
            515                 520                 525

Phe Gly Ala Phe Asn MET Val Tyr Trp Val Val Tyr Leu Ser Lys Asp Thr MET
530                 535                 540                 545

Glu Lys Ser Glu Ser Leu MET
        550
```

(2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 33 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

AGCCCGAATT CCATGGACGT TCTGGGCTGG CTG

(2) INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 29 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

GGTTTCCAAG CTTACTTTGG AGAGGTAGC

(2) INFORMATION FOR SEQ ID NO: 11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1555 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 47..1405

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

```
        10         20         30         40         49         58
GAATTCCCCA AGTTTGCGCG GACCCCGTCC CGAGCCCGCC GCGGCC ATG GAC GCG CCC GCC
                                                   >
                                                   MET Asp Ala Pro Ala

     67         76         85         94         103        112
CGG CTG CTG GCC CCG CTC CTG CTC CTC TGC GCG CAG CAG CTC CGC GGC ACC AGA
Arg Leu Leu Ala Pro Leu Leu Leu Leu Cys Ala Gln Gln Leu Arg Gly Thr Arg

    121        130        139        148        157        166
GCG ATG AAT GAC ATC GGC GAC TAC GTG GGC TCC AAC CTG GAG ATC TCC TGG CTC
Ala MET Asn Asp Ile Gly Asp Tyr Val Gly Ser Asn Leu Glu Ile Ser Trp Leu

    175        184        193        202        211        220
CCC AAC CTG GAC GGG CTG ATA GCC GGT TAC GCC CGC AAC TTC CGG CCT GGC ATC
Pro Asn Leu Asp Gly Leu Ile Ala Gly Tyr Ala Arg Asn Phe Arg Pro Gly Ile

    229        238        247        256        265        274
GGA GGC CCC CCC GTG AAT GTG GCC CTT GCC CTG GAG GTG GCC AGC ATC GAC CAC
Gly Gly Pro Pro Val Asn Val Ala Leu Ala Leu Glu Val Ala Ser Ile Asp His

    283        292        301        310        319        328
ATC TCA GAG GCC AAC ATG GAG TAC ACC ATG ACG GTG TTC CTG CAC CAG AGC TGG
Ile Ser Glu Ala Asn MET Glu Tyr Thr MET Thr Val Phe Leu His Gln Ser Trp

    337        346        355        364        373        382
CGG GAC AGC AGG CTC TCC TAC AAC CAC ACC AAC GAG ACC CTG GGC CTG GAC AGC
Arg Asp Ser Arg Leu Ser Tyr Asn His Thr Asn Glu Thr Leu Gly Leu Asp Ser

    391        400        409        418        427        436
CGC TTC GTG GAC AAG CTG TGG CTG CCC GAC ACC TTC ATC GTG AAC GCC AAG TCG
Arg Phe Val Asp Lys Leu Trp Leu Pro Asp Thr Phe Ile Val Asn Ala Lys Ser

    445        454        463        472        481        490
GCC TGG TTC CAC GAC GTG ACG GTG GAG AAC AAG CTC ATC CGG CTG CAG CCC GAC
Ala Trp Phe His Asp Val Thr Val Glu Asn Lys Leu Ile Arg Leu Gln Pro Asp

    499        508        517        526        535        544
GGG GTG ATC CTG TAC AGC ATC CGA ATC ACC TCC ACT GTG GCC TGC GAC ATG GAC
Gly Val Ile Leu Tyr Ser Ile Arg Ile Thr Ser Thr Val Ala Cys Asp MET Asp

    553        562        571        580        589        598
CTG GCC AAA TTC CCC ATG GAC GAG CAG GAG TGC ATG CTG GAC CTG GAG AGC TAC
Leu Ala Lys Phe Pro MET Asp Glu Gln Glu Cys MET Leu Asp Leu Glu Ser Tyr

    607        616        625        634        643        652
GGT TAC TCA TCG GAG GAC ATC GTC TAC TAC TGG TCG GAG AGC CAG GAG CAC ATC
Gly Tyr Ser Ser Glu Asp Ile Val Tyr Tyr Trp Ser Glu Ser Gln Glu His Ile

    661        670        679        688        697        706
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CAC GGG CTG GAC AAG CTG CAG CTG GCG CAG TTC ACC ATC ACC AGC TAC CGC TTC
His Gly Leu Asp Lys Leu Gln Leu Ala Gln Phe Thr Ile Thr Ser Tyr Arg Phe

    715         724         733         742         751         760

ACC ACG GAG CTG ATG AAC TTC AAG TCC GCT GGC CAG TTC CCA CGG CTC AGC CTG
Thr Thr Glu Leu MET Asn Phe Lys Ser Ala Gly Gln Phe Pro Arg Leu Ser Leu

    769         778         787         796         805         814

CAC TTC CAC CTG CGG AGG AAC CGC GGC GTG TAC ATC ATC CAA TCC TAC ATG CCC
His Phe His Leu Arg Arg Asn Arg Gly Val Tyr Ile Ile Gln Ser Tyr MET Pro

    823         832         841         850         859         868

TCC GTC CTG CTG GTC GCC ATG TCC TGG GTC TCC TTC TGG ATC AGC CAG GCG GCG
Ser Val Leu Leu Val Ala MET Ser Trp Val Ser Phe Trp Ile Ser Gln Ala Ala

    877         886         895         904         913         922

GTG CCC GCC AGG GTG TCT CTA GGC ATC ACC ACG GTG CTG ACG ATG ACC ACG CTC
Val Pro Ala Arg Val Ser Leu Gly Ile Thr Thr Val Leu Thr MET Thr Thr Leu

    931         940         949         958         967         976

ATG GTC AGT GCC CGC TCC TCC CTG CCA CGG GCA TCA GCC ATC AAG GCA CTG GAC
MET Val Ser Ala Arg Ser Ser Leu Pro Arg Ala Ser Ala Ile Lys Ala Leu Asp

    985         994        1003        1012        1021        1030

GTC TAC TTC TGG ATC TGC TAT GTC TTC GTG TTT GCC GCC CTG GTG GAG TAC GCC
Val Tyr Phe Trp Ile Cys Tyr Val Phe Val Phe Ala Ala Leu Val Glu Tyr Ala

   1039        1048        1057        1066        1075        1084

TTT GCT CAT TTC AAC GCC GAC TAC AGG AAG AAG CAG AAG GCC AAG GTC AAG GTC
Phe Ala His Phe Asn Ala Asp Tyr Arg Lys Lys Gln Lys Ala Lys Val Lys Val

   1093        1102        1111        1120        1129        1138

TCC AGG CCG AGG GCA GAG ATG GAC GTG AGG AAC GCC ATT GTC CTC TTC TCC CTC
Ser Arg Pro Arg Ala Glu MET Asp Val Arg Asn Ala Ile Val Leu Phe Ser Leu

   1147        1156        1165        1174        1183        1192

TCT GCT GCC GGC GTC ACG CAG GAG CTG GCC ATC TCC CGC CGG CAG CGC CGC GTC
Ser Ala Ala Gly Val Thr Gln Glu Leu Ala Ile Ser Arg Arg Gln Arg Arg Val

   1201        1210        1219        1228        1237        1246

CCG GGG AAC CTG ATG GGC TCC TAC AGG TCG GTG GGG GTG GAG ACA GGG GAG ACG
Pro Gly Asn Leu MET Gly Ser Tyr Arg Ser Val Gly Val Glu Thr Gly Glu Thr

   1255        1264        1273        1282        1291        1300

AAG AAG GAG GGG GCA GCC CGC TCA GGA GGC CAG GGG GGC ATC CGT GCC CGG CTC
Lys Lys Glu Gly Ala Ala Arg Ser Gly Gly Gln Gly Gly Ile Arg Ala Arg Leu

   1309        1318        1327        1336        1345        1354

AGG CCC ATC GAC GCA GAC ACC ATT GAC ATT TAC GCC CGC GCT GTG TTC CCT GCG
Arg Pro Ile Asp Ala Asp Thr Ile Asp Ile Tyr Ala Arg Ala Val Phe Pro Ala

   1363        1372        1381        1390        1399                1415
___ ___ ___ ___ ___ ___ ___ ___ ___ ___ ___ ___ ___ ___ ___ ___>
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GCG TTT GCG GCC GTC AAT GTC ATC TAC TGG GCG GCA TAC GCC ATG TGA GCACAGGACT
Ala Phe Ala Ala Val Asn Val Ile Tyr Trp Ala Ala Tyr Ala MET  .

      1425        1435        1445        1455        1465        1475

CAGGCCACCC TCGCTTGTCC TGGCGCCCGG CGGCAGCTGC CCAGAAACTT CCTGGGAGAA

1485        1495        1505        1515        1525        1535

AGAGCCCTCG GGCTGCCTTC CCCTCTGCGT GTTTCGAAGT GGGATGACAG TCGGCCACGG

1545        1555
AAAACAAGAG GAAGCCTCGG
```

(2) INFORMATION FOR SEQ ID NO: 12:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 452 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

```
MET Asp Ala Pro Ala Arg Leu Leu Ala Pro Leu Leu Leu Leu Cys Ala Gln Gln
1               5                  10                  15

Leu Arg Gly Thr Arg Ala MET Asn Asp Ile Gly Asp Tyr Val Gly Ser Asn Leu Glu
     20                  25                  30                  35

Ile Ser Trp Leu Pro Asn Leu Asp Gly Leu Ile Ala Gly Tyr Ala Arg Asn Phe Arg
         40                  45                  50                  55

Pro Gly Ile Gly Gly Pro Pro Val Asn Val Ala Leu Ala Leu Glu Val Ala Ser Ile
             60                  65                  70                  75

Asp His Ile Ser Glu Ala Asn MET Glu Tyr Thr MET Thr Val Phe Leu His Gln Ser
                 80                  85                  90

Trp Arg Asp Ser Arg Leu Ser Tyr Asn His Thr Asn Glu Thr Leu Gly Leu Asp Ser
95                  100                 105                 110

Arg Phe Val Asp Lys Leu Trp Leu Pro Asp Thr Phe Ile Val Asn Ala Lys Ser
    115                 120                 125                 130
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Ala Trp Phe His Asp Val Thr Val Glu Asn Lys Leu Ile Arg Leu Gln Pro Asp
          135                 140                 145

Gly Val Ile Leu Tyr Ser Ile Arg Ile Thr Ser Thr Val Ala Cys Asp MET Asp
150                 155                 160                 165

Leu Ala Lys Phe Pro MET Asp Glu Gln Glu Cys MET Leu Asp Leu Glu Ser Tyr
          170                 175                 180                 185

Gly Tyr Ser Ser Glu Asp Ile Val Tyr Tyr Trp Ser Glu Ser Gln Glu His Ile
                    190                 195                 200

His Gly Leu Asp Lys Leu Gln Leu Ala Gln Phe Thr Ile Thr Ser Tyr Arg Phe
     205                 210                 215                 220

Thr Thr Glu Leu MET Asn Phe Lys Ser Ala Gly Gln Phe Pro Arg Leu Ser Leu
          225                 230                 235

His Phe His Leu Arg Arg Asn Arg Gly Val Tyr Ile Ile Gln Ser Tyr MET Pro
240                 245                 250                 255

Ser Val Leu Leu Val Ala MET Ser Trp Val Ser Phe Trp Ile Ser Gln Ala Ala
          260                 265                 270                 275

Val Pro Ala Arg Val Ser Leu Gly Ile Thr Thr Val Leu Thr MET Thr Thr Leu
                    280                 285                 290

MET Val Ser Ala Arg Ser Ser Leu Pro Arg Ala Ser Ala Ile Lys Ala Leu Asp
     295                 300                 305                 310

Val Tyr Phe Trp Ile Cys Tyr Val Phe Val Phe Ala Ala Leu Val Glu Tyr Ala
          315                 320                 325

Phe Ala His Phe Asn Ala Asp Tyr Arg Lys Lys Gln Lys Ala Lys Val Lys Val
330                 335                 340                 345

Ser Arg Pro Arg Ala Glu MET Asp Val Arg Asn Ala Ile Val Leu Phe Ser Leu
          350                 355                 360                 365

Ser Ala Ala Gly Val Thr Gln Glu Leu Ala Ile Ser Arg Arg Gln Arg Arg Val
                    370                 375                 380

Pro Gly Asn Leu MET Gly Ser Tyr Arg Ser Val Gly Val Glu Thr Gly Glu Thr
     385                 390                 395                 400

Lys Lys Glu Gly Ala Ala Arg Ser Gly Gly Gln Gly Gly Ile Arg Ala Arg Leu
          405                 410                 415

Arg Pro Ile Asp Ala Asp Thr Ile Asp Ile Tyr Ala Arg Ala Val Phe Pro Ala
420                 425                 430                 435

Ala Phe Ala Ala Val Asn Val Ile Tyr Trp Ala Ala Tyr Ala MET  .
          440                 445                 450
```

(2) INFORMATION FOR SEQ ID NO: 13:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 30 base pairs

(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

TAATGAGTTT AAACCATAGC TTCTTCCAGT

(2) INFORMATION FOR SEQ ID NO: 14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:

CATGATGGAT CCGCCCGCTC AGAC

CLAIMS:

1. A stably co-transfected eukaryotic cell line capable of expressing a human $GABA_A$ receptor, which receptor comprises the alpha-4 receptor subunit, at least one beta receptor subunit and the delta receptor subunit.

2. A stably co-transfected eukaryotic cell line capable of expressing a human $GABA_A$ receptor, which receptor comprises the alpha-4 receptor subunit, at least one beta receptor subunit and at least one gamma receptor subunit.

3. A stably co-transfected eukaryotic cell line capable of expressing a human $GABA_A$ receptor, which receptor comprises at least one alpha receptor subunit, at least one beta receptor subunit and the delta receptor subunit.

4. A cell line as claimed in any one of claims 1 to 3 wherein the cell line is a rodent fibroblast cell line.

5. A process for the preparation of a eukaryotic cell line capable of expressing a human $GABA_A$ receptor, which comprises stably co-transfecting a rodent fibroblast host cell with at least three expression vectors, one such vector harbouring the human cDNA sequence encoding the alpha-4 receptor subunit, another such vector harbouring the human cDNA sequence encoding a beta receptor subunit, and a third such vector harbouring the human cDNA sequence encoding the delta receptor subunit.

6. A process for the preparation of a eukaryotic cell line capable of expressing a human $GABA_A$ receptor, which comprises stably co-transfecting a rodent fibroblast host cell with at least three expression vectors, one such vector harbouring the human cDNA sequence encoding the alpha-4 receptor subunit, another such vector harbouring the human cDNA sequence encoding a beta receptor subunit, and a third such vector harbouring the human cDNA sequence encoding a gamma receptor subunit.

7. A process for the preparation of a eukaryotic cell line capable of expressing a human $GABA_A$ receptor, which comprises stably co-transfecting a rodent fibroblast host cell with at least three expression vectors, one such vector harbouring the human cDNA sequence encoding an alpha receptor subunit, another such vector harbouring the human cDNA sequence encoding a beta receptor subunit, and a third such vector harbouring the human cDNA sequence encoding the delta receptor subunit.

8. A process as claimed in any one of claims 5 to 7 wherein the eukaryotic cell line is a rodent fibroblast cell line.

9. A DNA molecule encoding the α_4 subunit of the human $GABA_A$ receptor comprising all or a portion of the sequence depicted in Figure 2 herein SEQ. ID. NO.: 7.

10. A DNA molecule encoding the δ subunit of the human $GABA_A$ receptor comprising all or a portion of the sequence depicted in Figure 3 herein SEQ. ID. NO.: 10.

11. A recombinant expression vector comprising the nucleotide sequence of the human α_4 $GABA_A$ receptor subunit together with additional sequences capable of directing the synthesis of the said human α_4 $GABA_A$ receptor subunit in cultures of stably co-transfected eukaryotic cells.

12. A recombinant expression vector comprising the nucleotide sequence of the human δ $GABA_A$ receptor subunit together with additional sequences capable of directing the synthesis of the said human δ $GABA_A$ receptor subunit in cultures of stably co-transfected eukaryotic cells.

13. A protein preparation of human $GABA_A$ receptor subunit combinations comprising the human α_4 $GABA_A$ receptor subunit derived from a culture of stably co-transfected eukaryotic cells.

14. A protein preparation of human $GABA_A$ receptor subunit combinations comprising the human δ $GABA_A$ receptor subunit derived from a culture of stably co-transfected eukaryotic cells.

15. A membrane preparation containing $GABA_A$ receptor subunit combinations comprising the human α_4 $GABA_A$ receptor subunit derived from a culture of stably co-transfected eukaryotic cells.

16. A membrane preparation containing $GABA_A$ receptor subunit combinations comprising the human δ $GABA_A$ receptor subunit derived from a culture of stably co-transfected eukaryotic cells.

17. A preparation as claimed in claim 13 or 14 wherein the subunit combination derived is the $\alpha_4\beta_3\delta$ subunit combination of the human $GABA_A$ receptor.

18. A preparation as claimed in claim 13 wherein the subunit combination derived is the $\alpha_4\beta_3\gamma_2$ subunit combination of the human $GABA_A$ receptor.

19. A preparation as claimed in claim 13 wherein the subunit combination derived is the $\alpha_4\beta_2\gamma_2$ subunit combination of the human $GABA_A$ receptor.

20. A preparation as claimed in claim 14 wherein the subunit combination derived is the $\alpha_6\beta_3\delta$ subunit combination of the human $GABA_A$ receptor.

21. A preparation as claimed in claim 15 or 16 wherein the subunit combination derived is the $\alpha_4\beta_3\delta$ subunit combination of the human $GABA_A$ receptor.

22. A preparation as claimed in claim 15 wherein the subunit combination derived is the $\alpha_4\beta_3\gamma_2$ subunit combination of the human $GABA_A$ receptor.

23. A preparation as claimed in claim 15 wherein the subunit combination derived is the $\alpha_4\beta_2\gamma_2$ subunit combination of the human $GABA_A$ receptor.

24. A preparation as claimed in claim 16 wherein the subunit combination derived is the $\alpha_6\beta_3\delta$ subunit combination of the human $GABA_A$ receptor.

25. The use of the cell line as claimed in any one of claims 1 to 3, and membrane preparations derived therefrom, in screening for and designing medicaments which act upon the human $GABA_A$ receptor.

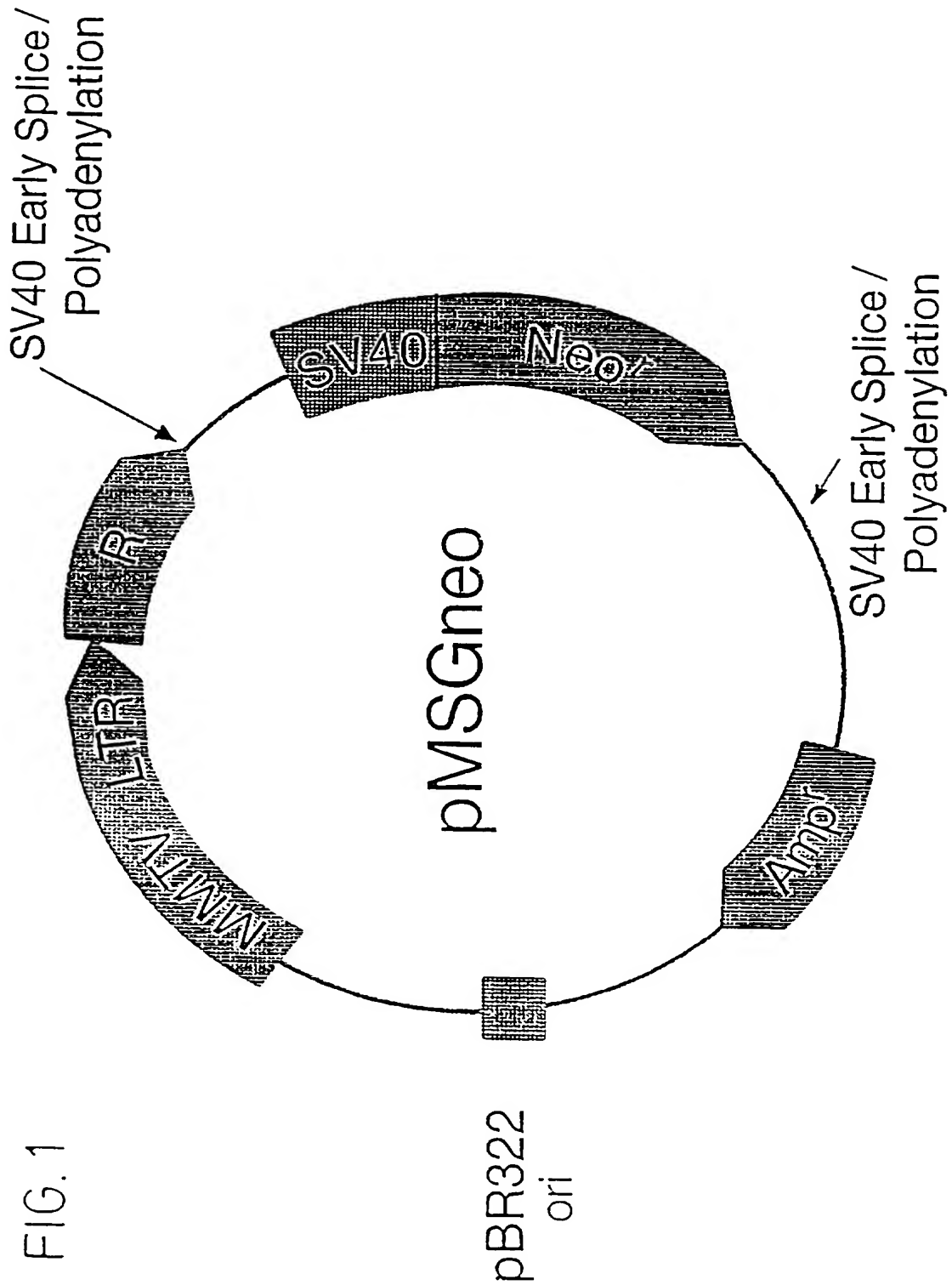
SV40 Early Splice / Polyadenylation
SV40 Neo^r
SV40 Early Splice / Polyadenylation
pMSGneo
LTR
MMTV
Amp^r
pBR322 ori

FIG. 1

FIGURE 2

```
          10          20          30          41          50          59
GGATCCGTGA ACAGCTTGAA GTATGGCATG TTGCAAAG  >ATG GTT TCT GCC AAG AAG GTA
                                           MET Val Ser Ala Lys Lys Val

          68          77          86          95          104         113
CCC GCG ATC ACT CTG TCC GCC GGG GTC AGT TTC GCC CTC CTG CGC TTC CTG TGC
Pro Ala Ile Thr Leu Ser Ala Gly Val Ser Phe Ala Leu Leu Arg Phe Leu Cys

          122         131         140         149         158         167
CTG GCG GTT TGT TTA AAC GAA TCC CCA GGA CAG AAC CAA AAG GAG GAG AAA TTG
Leu Ala Val Cys Leu Asn Glu Ser Pro Gly Gln Asn Gln Lys Glu Glu Lys Leu

          176         185         194         203         212         221
TGC ACA GAA AAT TTC ACC CGC ATC CTG GAC AGT TTG CTC GAT GGT TAT GAC AAC
Cys Thr Glu Asn Phe Thr Arg Ile Leu Asp Ser Leu Leu Asp Gly Tyr Asp Asn

          230         239         248         257         266         275
AGG CTG CGT CCT GGA TTT GGG GGT CCT GTT ACA GAA GTG AAA ACT GAC ATA TAT
Arg Leu Arg Pro Gly Phe Gly Gly Pro Val Thr Glu Val Lys Thr Asp Ile Tyr

          284         293         302         311         320         329
GTC ACC AGC TTT GGA CCT GTT TCT GAT GTT GAA GTG GAA TAC ACA ATG GAT GTG
Val Thr Ser Phe Gly Pro Val Ser Asp Val Glu Val Glu Tyr Thr MET Asp Val

          338         347         356         365         374         383
TTC TTC AGG CAG ACA TGG ATT GAC AAA AGA TTA AAA TAT GAC GGC CCC ATT GAA
Phe Phe Arg Gln Thr Trp Ile Asp Lys Arg Leu Lys Tyr Asp Gly Pro Ile Glu

          392         401         410         419         428         437
ATT TTG AGA TTG AAC AAT ATG ATG GTA ACG AAA GTG TGG ACC CCT GAT ACT TTC
Ile Leu Arg Leu Asn Asn MET MET Val Thr Lys Val Trp Thr Pro Asp Thr Phe

          446         455         464         473         482         491
TTC AGG AAT GGA AAG AAA TCT GTC TCA CAT AAT ATG ACA GCT CCA AAT AAG CTT
Phe Arg Asn Gly Lys Lys Ser Val Ser His Asn MET Thr Ala Pro Asn Lys Leu

          500         509         518         527         536         545
TTT AGA ATT ATG AGA AAT GGT ACT ATT TTA TAC ACA ATG AGA CTC ACC ATA AGT
Phe Arg Ile MET Arg Asn Gly Thr Ile Leu Tyr Thr MET Arg Leu Thr Ile Ser

          554         563         572         581         590         599
GCG GAG TGT CCC ATG AGA TTG GTG GAT TTT CCC ATG GAT GGT CAT GCA TGC CCT
Ala Glu Cys Pro MET Arg Leu Val Asp Phe Pro MET Asp Gly His Ala Cys Pro

          608         617         626         635         644         653
GTG AAA TTC GGG AGT TAT GCC TAT CCA AAG AGT GAG ATG ATC TAT ACC TGG ACA
Val Lys Phe Gly Ser Tyr Ala Tyr Pro Lys Ser Glu MET Ile Tyr Thr Trp Thr
```

FIGURE 2 (CONTINUED)

```
        662         671         680         689         698         707
AAA GGT CCT GAG AAA TCA GTT GAA GTT CCG AAG GAG TCT TCC AGC TTA GTT CAA
Lys Gly Pro Glu Lys Ser Val Glu Val Pro Lys Glu Ser Ser Ser Leu Val Gln

        716         725         734         743         752         761
TAT GAT TTG ATT GGG CAA ACC GTA TCA AGT GAA ACC ATC AAA TCA ATT ACG GGT
Tyr Asp Leu Ile Gly Gln Thr Val Ser Ser Glu Thr Ile Lys Ser Ile Thr Gly

        770         779         788         797         806         815
GAA TAT ATT GTT ATG ACG GTT TAC TTC CAC CTC AGA CGG AAG ATG GGT TAT TTT
Glu Tyr Ile Val MET Thr Val Tyr Phe His Leu Arg Arg Lys MET Gly Tyr Phe

        824         833         842         851         860         869
ATG ATT CAG ACC TAT ATT CCG TGC ATT ATG ACA GTG ATT CTT TCT CAA GTT TCA
MET Ile Gln Thr Tyr Ile Pro Cys Ile MET Thr Val Ile Leu Ser Gln Val Ser

        878         887         896         905         914         923
TTT TGG ATA AAT AAA GAA TCA GTT CCC GCT AGG ACC GTA TTT GGA ATA ACA ACT
Phe Trp Ile Asn Lys Glu Ser Val Pro Ala Arg Thr Val Phe Gly Ile Thr Thr

        932         941         950         959         968         977
GTC CTC ACC ATG ACC ACA CTA AGC ATC AGT GCA CGA CAT TCT TTG CCC AAA GTG
Val Leu Thr MET Thr Thr Leu Ser Ile Ser Ala Arg His Ser Leu Pro Lys Val

        986         995        1004        1013        1022        1031
TCC TAT GCT ACC GCC ATG GAC TGG TTC ATA GCT GTC TGC TTT GCT TTT GTA TTT
Ser Tyr Ala Thr Ala MET Asp Trp Phe Ile Ala Val Cys Phe Ala Phe Val Phe

       1040        1049        1058        1067        1076        1085
TCG GCC CTT ATC GAG TTT GCT GCT GTC AAC TAT TTC ACC AAT ATT CAA ATG GAA
Ser Ala Leu Ile Glu Phe Ala Ala Val Asn Tyr Phe Thr Asn Ile Gln MET Glu

       1094        1103        1112        1121        1130        1139
AAA GCC AAA AGG AAG ACA TCA AAG CCC CCT CAG GAA GTT CCC GCT GCT CCA GTG
Lys Ala Lys Arg Lys Thr Ser Lys Pro Pro Gln Glu Val Pro Ala Ala Pro Val

       1148        1157        1166        1175        1184        1193
CAG AGA GAG AAG CAT CCT GAA GCC CCT CTG CAG AAT ACA AAT GCC AAT TTG AAC
Gln Arg Glu Lys His Pro Glu Ala Pro Leu Gln Asn Thr Asn Ala Asn Leu Asn

       1202        1211        1220        1229        1238        1247
ATG AGA AAA AGA ACA AAT GCT TTG GTT CAC TCT GAA TCT GAT GTT GGC AAC AGA
MET Arg Lys Arg Thr Asn Ala Leu Val His Ser Glu Ser Asp Val Gly Asn Arg

       1256        1265        1274        1283        1292        1301
ACT GAG GTG GGA AAC CAT TCA AGC AAA TCT TCC ACA GTT GTT CAA GAA TCT TCT
Thr Glu Val Gly Asn His Ser Ser Lys Ser Ser Thr Val Val Gln Glu Ser Ser

       1310        1319        1328        1337        1346        1355
AAA GGC ACA CCT CGG TCT TAC TTA GCT TCC AGT CCA AAC CCA TTC AGC CGT GCA
Lys Gly Thr Pro Arg Ser Tyr Leu Ala Ser Ser Pro Asn Pro Phe Ser Arg Ala
```

FIGURE 2 (CONTINUED)

```
      1364        1373        1382        1391        1400        1409
AAT GCA GCT GAA ACC ATA TCT GCA GCA AGA GCA CTT CCA TCT GCT TCT CCT ACT
Asn Ala Ala Glu Thr Ile Ser Ala Ala Arg Ala Leu Pro Ser Ala Ser Pro Thr

      1418        1427        1436        1445        1454        1463
TCT ATC CGA ACT GGA TAT ATG CCT CGA AAG GCT TCA GTT GGA TCT GCT TCT ACT
Ser Ile Arg Thr Gly Tyr MET Pro Arg Lys Ala Ser Val Gly Ser Ala Ser Thr

      1472        1481        1490        1499        1508        1517
CGT CAC GTG TTT GGA TCA AGA CTG CAG AGG ATA AAG ACC ACA GTT AAT ACC ATA
Arg His Val Phe Gly Ser Arg Leu Gln Arg Ile Lys Thr Thr Val Asn Thr Ile

      1526        1535        1544        1553        1562        1571
GGG GCT ACT GGG AAG TTG TCA GCT ACT CCT CCT CCA TCG GCT CCA CCA CCT TCT
Gly Ala Thr Gly Lys Leu Ser Ala Thr Pro Pro Pro Ser Ala Pro Pro Pro Ser

      1580        1589        1598        1607        1616        1625
GGA TCT GGC ACA AGT AAA ATA GAC AAA TAT GCC CGT ATT CTC TTT CCA GTC ACA
Gly Ser Gly Thr Ser Lys Ile Asp Lys Tyr Ala Arg Ile Leu Phe Pro Val Thr

      1634        1643        1652        1661        1670        1679
TTT GGG GCA TTT AAC ATG GTT TAT TGG GTT GTT TAT TTA TCT AAG GAC ACT ATG
Phe Gly Ala Phe Asn MET Val Tyr Trp Val Val Tyr Leu Ser Lys Asp Thr MET

      1688        1697        1707
                              >
GAG AAA TCA GAA AGT CTA ATG TGA ATTC
Glu Lys Ser Glu Ser Leu MET  .
```

Figure 3

```
          10          20          30          40          49          58
GAATTCCCCA AGTTTGCGCG GACCCCGTCC CGAGCCCGCC GCGGCC ATG GAC GCG CCC GCC
                                                   >  MET Asp Ala Pro Ala

      67          76          85          94         103         112
CGG CTG CTG GCC CCG CTC CTG CTC CTC TGC GCG CAG CAG CTC CGC GGC ACC AGA
Arg Leu Leu Ala Pro Leu Leu Leu Leu Cys Ala Gln Gln Leu Arg Gly Thr Arg

     121         130         139         148         157         166
GCG ATG AAT GAC ATC GGC GAC TAC GTG GGC TCC AAC CTG GAG ATC TCC TGG CTC
Ala MET Asn Asp Ile Gly Asp Tyr Val Gly Ser Asn Leu Glu Ile Ser Trp Leu

     175         184         193         202         211         220
CCC AAC CTG GAC GGG CTG ATA GCC GGT TAC GCC CGC AAC TTC CGG CCT GGC ATC
Pro Asn Leu Asp Gly Leu Ile Ala Gly Tyr Ala Arg Asn Phe Arg Pro Gly Ile

     229         238         247         256         265         274
GGA GGC CCC CCC GTG AAT GTG GCC CTT GCC CTG GAG GTG GCC AGC ATC GAC CAC
Gly Gly Pro Pro Val Asn Val Ala Leu Ala Leu Glu Val Ala Ser Ile Asp His

     283         292         301         310         319         328
ATC TCA GAG GCC AAC ATG GAG TAC ACC ATG ACG GTG TTC CTG CAC CAG AGC TGG
Ile Ser Glu Ala Asn MET Glu Tyr Thr MET Thr Val Phe Leu His Gln Ser Trp

     337         346         355         364         373         382
CGG GAC AGC AGG CTC TCC TAC AAC CAC ACC AAC GAG ACC CTG GGC CTG GAC AGC
Arg Asp Ser Arg Leu Ser Tyr Asn His Thr Asn Glu Thr Leu Gly Leu Asp Ser

     391         400         409         418         427         436
CGC TTC GTG GAC AAG CTG TGG CTG CCC GAC ACC TTC ATC GTG AAC GCC AAG TCG
Arg Phe Val Asp Lys Leu Trp Leu Pro Asp Thr Phe Ile Val Asn Ala Lys Ser

     445         454         463         472         481         490
GCC TGG TTC CAC GAC GTG ACG GTG GAG AAC AAG CTC ATC CGG CTG CAG CCC GAC
Ala Trp Phe His Asp Val Thr Val Glu Asn Lys Leu Ile Arg Leu Gln Pro Asp

     499         508         517         526         535         544
GGG GTG ATC CTG TAC AGC ATC CGA ATC ACC TCC ACT GTG GCC TGC GAC ATG GAC
Gly Val Ile Leu Tyr Ser Ile Arg Ile Thr Ser Thr Val Ala Cys Asp MET Asp

     553         562         571         580         589         598
CTG GCC AAA TTC CCC ATG GAC GAG CAG GAG TGC ATG CTG GAC CTG GAG AGC TAC
Leu Ala Lys Phe Pro MET Asp Glu Gln Glu Cys MET Leu Asp Leu Glu Ser Tyr

     607         616         625         634         643         652
GGT TAC TCA TCG GAG GAC ATC GTC TAC TAC TGG TCG GAG AGC CAG GAG CAC ATC
Gly Tyr Ser Ser Glu Asp Ile Val Tyr Tyr Trp Ser Glu Ser Gln Glu His Ile
```

Figure 3 (continued)

```
     661         670         679         688         697         706
CAC GGG CTG GAC AAG CTG CAG CTG GCG CAG TTC ACC ATC ACC AGC TAC CGC TTC
His Gly Leu Asp Lys Leu Gln Leu Ala Gln Phe Thr Ile Thr Ser Tyr Arg Phe

     715         724         733         742         751         760
ACC ACG GAG CTG ATG AAC TTC AAG TCC GCT GGC CAG TTC CCA CGG CTC AGC CTG
Thr Thr Glu Leu MET Asn Phe Lys Ser Ala Gly Gln Phe Pro Arg Leu Ser Leu

     769         778         787         796         805         814
CAC TTC CAC CTG CGG AGG AAC CGC GGC GTG TAC ATC ATC CAA TCC TAC ATG CCC
His Phe His Leu Arg Arg Asn Arg Gly Val Tyr Ile Ile Gln Ser Tyr MET Pro

     823         832         841         850         859         868
TCC GTC CTG CTG GTC GCC ATG TCC TGG GTC TCC TTC TGG ATC AGC CAG GCG GCG
Ser Val Leu Leu Val Ala MET Ser Trp Val Ser Phe Trp Ile Ser Gln Ala Ala

     877         886         895         904         913         922
GTG CCC GCC AGG GTG TCT CTA GGC ATC ACC ACG GTG CTG ACG ATG ACC ACG CTC
Val Pro Ala Arg Val Ser Leu Gly Ile Thr Thr Val Leu Thr MET Thr Thr Leu

     931         940         949         958         967         976
ATG GTC AGT GCC CGC TCC TCC CTG CCA CGG GCA TCA GCC ATC AAG GCA CTG GAC
MET Val Ser Ala Arg Ser Ser Leu Pro Arg Ala Ser Ala Ile Lys Ala Leu Asp

     985         994        1003        1012        1021        1030
GTC TAC TTC TGG ATC TGC TAT GTC TTC GTG TTT GCC GCC CTG GTG GAG TAC GCC
Val Tyr Phe Trp Ile Cys Tyr Val Phe Val Phe Ala Ala Leu Val Glu Tyr Ala

    1039        1048        1057        1066        1075        1084
TTT GCT CAT TTC AAC GCC GAC TAC AGG AAG AAG CAG AAG GCC AAG GTC AAG GTC
Phe Ala His Phe Asn Ala Asp Tyr Arg Lys Lys Gln Lys Ala Lys Val Lys Val

    1093        1102        1111        1120        1129        1138
TCC AGG CCG AGG GCA GAG ATG GAC GTG AGG AAC GCC ATT GTC CTC TTC TCC CTC
Ser Arg Pro Arg Ala Glu MET Asp Val Arg Asn Ala Ile Val Leu Phe Ser Leu

    1147        1156        1165        1174        1183        1192
TCT GCT GCC GGC GTC ACG CAG GAG CTG GCC ATC TCC CGC CGG CAG CGC CGC GTC
Ser Ala Ala Gly Val Thr Gln Glu Leu Ala Ile Ser Arg Arg Gln Arg Arg Val

    1201        1210        1219        1228        1237        1246
CCG GGG AAC CTG ATG GGC TCC TAC AGG TCG GTG GGG GTG GAG ACA GGG GAG ACG
Pro Gly Asn Leu MET Gly Ser Tyr Arg Ser Val Gly Val Glu Thr Gly Glu Thr

    1255        1264        1273        1282        1291        1300
AAG AAG GAG GGG GCA GCC CGC TCA GGA GGC CAG GGG GGC ATC CGT GCC CGG CTC
Lys Lys Glu Gly Ala Ala Arg Ser Gly Gly Gln Gly Gly Ile Arg Ala Arg Leu
```

Figure 3 (continued)

```
    1309        1318        1327        1336        1345        1354
AGG CCC ATC GAC GCA GAC ACC ATT GAC ATT TAC GCC CGC GCT GTG TTC CCT GCG
Arg Pro Ile Asp Ala Asp Thr Ile Asp Ile Tyr Ala Arg Ala Val Phe Pro Ala

    1363        1372        1381        1390        1399            1415
GCG TTT GCG GCC GTC AAT GTC ATC TAC TGG GCG GCA TAC GCC ATG TGA> GCACAGGACT
Ala Phe Ala Ala Val Asn Val Ile Tyr Trp Ala Ala Tyr Ala MET  .

      1425        1435        1445        1455        1465        1475        1485
CAGGCCACCC TCGCTTGTCC TGGCGCCCGG CGGCAGCTGC CCAGAAACTT CCTGGGAGAA AGAGCCCTCG

      1495        1505        1515        1525        1535        1545        1555
GGCTGCCTTC CCCTCTGCGT GTTTCGAAGT GGGATGACAG TCGGCCACGG AAAACAAGAG GAAGCCTCGG
```

INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 95/02323

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/12 C07K14/705 C12N5/10

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US,A,5 166 066 (CARTER DONALD B) 24 November 1992 see page 1, column 1, line 24 - page 1, column 1, line 30 see page 3, column 1, line 54 - page 4, column 1, line 55 see page 6, column 1, line 31 - page 6, column 1, line 41; claims 1-20	3,4,7,8, 10,12, 14,16,25
A	WO,A,94 13799 (MERCK SHARP & DOHME ;HADINGHAM KAREN LOUISE (GB); WHITING PAUL JOH) 23 June 1994 see the whole document	1-25
A	WO,A,92 22652 (MERCK SHARP & DOHME) 23 December 1992 see the whole document	1-25

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

1

Date of the actual completion of the international search	Date of mailing of the international search report
7 February 1996	27. 02. 96

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax (+ 31-70) 340-3016	Nauche, S

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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A	FEBS LETT, SEP 9 1991, 289 (2) P227-30, NETHERLANDS, WISDEN W ET AL 'Cloning, pharmacological characteristics and expression pattern of the rat GABAA receptor alpha 4 subunit.' ---	
A	FEBS LETT, NOV 20 1989, 258 (1) P119-22, NETHERLANDS, YMER S ET AL 'Sequence and expression of a novel GABAA receptor alpha subunit.' ---	
A	J BIOL CHEM, APR 29 1994, 269 (17) P12993-8, UNITED STATES, TOGEL M ET AL 'gamma-Aminobutyric acidA receptors displaying association of gamma 3-subunits with beta 2/3 and different alpha-subunits exhibit unique pharmacological properties.' ---	
A	BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, vol. 167, no. 1, 28 February 1990 ORLANDO, FL US, pages 174-182, ZHAO-YANG ZHAO ET AL; 'Isolation of distantly related members in a multigene family using the polymerase chain reaction technique' ---	
A	CURRENT OPINION IN NEUROBIOLOGY, vol. 2, no. 3, June 1992 pages 263-269, WISDEN, W.; SEEBURG, P.H. 'GABA(a) receptor channels: from subunits to functional entities' see abstract -----	

1

INTERNATIONAL SEARCH REPORT

nformation on patent family members

International Application No
PCT/GB 95/02323

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
US-A-5166066	24-11-92	NONE		
WO-A-9413799	23-06-94	AU-B-	5655494	04-07-94
		CA-A-	2151236	23-06-94
		EP-A-	0673419	27-09-95
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		AU-B-	1921192	12-01-93
		CA-A-	2109193	12-12-92
		EP-A-	0589930	06-04-94
		JP-T-	6508023	14-09-94

DERWENT-ACC-NO: 1996-209359

DERWENT-WEEK: 200703

TITLE: DNA encoding alpha-4 and delta subunit (s) of the human GABAa receptor also stably co-transfected eukaryotic cells expressing receptors contg. these subunit (s), used for screening and designing drugs

INVENTOR: LE BOURDELLES B; WHITING P J

PATENT-ASSIGNEE: MERCK SHARP & DOHME LTD[MERI]

PRIORITY-DATA: 1994GB-020010 (October 1, 1994)

PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE
WO 9610637 A1	April 11, 1996	EN
EP 783576 A1	July 16, 1997	EN
JP 10506534 W	June 30, 1998	JA
US 6455276 B1	September 24, 2002	EN
US 20030013158 A1	January 16, 2003	EN
US 7157249 B2	January 2, 2007	EN

DESIGNATED-STATES: CA JP US AT BE CH DE DK ES FR GB GR IE IT LU MC NL PT SE AT BE CH DE DK ES FR GB GR IE IT LI LU NL PT SE

APPLICATION-DATA:

PUB-NO	APPL-DESCRIPTOR	APPL-NO	APPL-DATE
WO1996010637A1	N/A	1995WO-GB02323	September 29, 1995
EP 783576A1	N/A	1995EP-932842	September 29, 1995
EP 783576A1	N/A	1995WO-GB02323	September 29, 1995
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JP 10506534W	N/A	1996JP-511525	September 29, 1995
US 6455276B1	N/A	1997US-809802	June 19, 1997
US20030013158A1	N/A	2002US-211673	August 2, 2002
US 7157249B2	Based on	2002US-211673	August 2, 2002

INT-CL-CURRENT:

TYPE	IPC DATE
CIPP	C07H21/04 20060101
CIPP	C12N15/09 20060101
CIPS	C07K14/705 20060101
CIPS	C07K14/705 20060101
CIPS	C07K14/715 20060101
CIPS	C12N15/12 20060101
CIPS	C12N15/63 20060101
CIPS	C12N5/10 20060101
CIPS	C12N5/16 20060101

ABSTRACTED-PUB-NO: WO 9610637 A1

BASIC-ABSTRACT:

New stably co-transfected eukaryotic cells are able to express a human GABAa receptor consisting of: (a) the ? 4, ? one ? and the ? subunits; (b) the ?4, ? one ? and ? one ? subunits; or (c) ? one ?, ? one ? and the ? subunits.

USE - The co-transfected cell lines and membrane prepns. are used to screen for, or design, subtype-specific drugs that act on human GABAa receptors (claimed), e.g. benzodiazepines, barbiturates, ?-carbolines and neurosteroids.

ADVANTAGE - Construction of recombinant receptors contg. the ?4 and ? subunit becomes possible for the first time.

TITLE-TERMS: DNA ENCODE ALPHA DELTA HUMAN RECEPTOR STABILISED CO TRANSFECTED EUKARYOTIC CELL EXPRESS CONTAIN SCREEN DESIGN DRUG

ADDL-INDEXING-TERMS: GAMMA AMINO BUTYRIC ACID

DERWENT-CLASS: B04 D16

CPI-CODES: B04-E02D; B04-E08; B04-F0200E; B11-C08E4; B12-K04F; D05-H09; D05-H12A; D05-H12E; D05-H14B2; D05-H17A4;

CHEMICAL-CODES: Chemical Indexing M1 *01* Fragmentation Code M423 M710 N102 N135 N136 N137 P831 Q233 V752 V753 V754

Chemical Indexing M6 *02* Fragmentation Code P831 Q233 R515 R521 R537 R614 R627 R633 R639

SECONDARY-ACC-NO:

CPI Secondary Accession Numbers: 1996-066806